

## REMARKS

Claims 3 and 5-15 are pending. All of the pending claims are rejected. Upon entry of the instant Amendment and Response, claims 3, 5, 6, and 8-17 will be pending. As a preliminary matter, Applicants appreciate the in person interview on August 18, 2009 between the Examiner, Dr. Pierre Belhumeur and Applicants' representative J. David Smith. Issues regarding patentability were discussed, and Applicants proposed the changes to claim 3 that are provided in the present Amendment and Response. That is, Applicants clarify that the claimed method is "a method of evaluating the efficiency of a sterilization process *on prion proteins*. Further, Applicants clarify that step a) involves "subjecting a sufficient amount of at least one prion protein degradation indicator in a container to said sterilization process *such that degradation of prion proteins occurs*." Also, Applicants clarify that step b) involves "determining the level of degradation of the *prion* indicator." Similarly, claim 5 was amended to reflect the amendments made to claim 3 to prevent a future rejection. No issue of new matter arises as there is inherent support throughout the specification as the Examiner recognizes. Also, Applicants add new claims 16 and 17 to specifically recite that the sterilization process includes ozone treatment in some embodiments and to specifically recite that determining the level of degradation of the prion protein degradation indicator is performed by Western Blot analysis in some embodiments. No issue of new matter arises from these claims since express support may be found in the embodiments exemplified in the specification. Applicants further cancel claim 7 without prejudice.

Applicants submit for the record a copy of the corresponding European Patent (Exhibit A), issued on June 6, 2007 from the corresponding European patent application.

### *Rejection under 35 USC 112, second paragraph*

The Examiner rejects claims 3 and 5-15 as allegedly unclear for the following reasons:

1. Claim 3 recites "A sterilization process" but should be changed to read "A method for evaluating the efficiency of a sterilization process *on prion proteins* comprising....," and
2. Claim 7 recites that the indicator is a biological indicator, a biochemical indicator or a chemical indicator and depends from claim 3 that recites that the indicator is

selected from SUP35, URE2 or HET-s. It is unclear whether these yeast prions are considered biological, biochemical and/or chemical while the specification teaches that biological indicators are usually bacterial spores.

Regarding 1), Applicants make the suggested change herein. Regarding 2), Applicants herein cancel the claim 7 without prejudice thereby obviating the rejection.

***Rejection under 35 USC 103***

A. The Examiner rejects claims 3 and 5-15 as allegedly unpatentable over Safar *et al.*, *Protein Science*, 1993, 2:2206-2216 in view of Coustou *et al.*, *PNAS* (1997), Glover *et al.*, *Cell* (1997) or Wickner, *Science* (1994). The Examiner admits that Safar *et al.* do not teach using Sup35p, Ure2p or Het-s protein. However, the Examiner relies upon each of Coustou *et al.*, *PNAS* (1997), Glover *et al.*, *Cell* (1997) or Wickner, *Science* (1994) for teaching one individually. The Examiner adds that one of ordinary skill in the art would have recognized that yeast prion analogs have the same property as mammalian prion proteins, and thus would be suitable to replace the mammalian prion proteins as an indicator. The Examiner further admits that the references, even in combination, do not teach the amount of indicator being 0.1 ng to 100 g, the Examiner says that the amount used by Safar *et al.* may be easily calculated as 140 µg. Further, the Examiner says that it is well recognized that only those gene products of yeast in amyloid or amyloid-like form are considered as prion counterparts (*See*, claim 3, amended to read “and is in amyloid form...”).

B. The Examiner rejects claims 3 and 9 as allegedly unpatentable over Safar *et al.*, *Protein Science*, 1993, 2:2206-2216 in view of Coustou *et al.*, *PNAS* (1997), Glover *et al.*, *Cell* (1997) or Wickner, *Science* (1994) and further in view of Feldman *et al.*, “Compatibility of medical devices and material with low-temperature hydrogen peroxide gas plasma,” (1997). The Examiner admits that the primary and secondary references do not teach using low temperature gas plasma or oxidizing sterilizing agents. However, allegedly Feldman *et al.* teach using a sterilization process to inactivate a prion using oxidizing agents such as hydrogen peroxide as a

form of low-temperature gas plasma (*citing* Column 30, line 33 through Column 34, line 42). Therefore, the Examiner says that it would have been obvious to substitute the sterilization technique of Feldman *et al.* for that used by the primary reference (Safar *et al.*), and the motivation to do so comes from the potential damage and safety concerns of the sterilization techniques of the primary and secondary references.

C. The Examiner rejects claims 3, 9, 10 and 13 as allegedly unpatentable over Safar *et al.*, *Protein Science*, 1993, 2:2206-2216 in view of Coustou *et al.*, *PNAS* (1997), Glover *et al.*, *Cell* (1997) or Wickner, *Science* (1994) and further in view of Dresdner *et al.*, U.S. Patent 5,357,636.

The Examiner admits that the primary and secondary references do not teach ozone-based exposure or sodium hydroxide as chemical exposure. However, the Examiner says that Dresdner *et al.* teach ozone-based exposure or sodium hydroxide as an antiseptic composition and that one of ordinary skill in the art would recognize this as an equivalent sterilization technique to the sterilization techniques of the primary reference (Safar *et al.*). The Examiner further admits that the primary and secondary references do not teach a porous, permeable, or semi-permeable container. However, the Examiner says that Dresdner *et al.* teach a porous and liquid-permeable medical glove for sterilization. The Examiner adds that it would have been obvious to replace a glass container of the primary reference with a porous medical glove, and that the motivation to make the modification comes from the fact that prions occur in various materials and various materials should be sterilized. Moreover, there is allegedly a reasonable expectation of success because various materials are indeed routinely sterilized.

#### **Response to Applicants' last arguments**

The Examiner understands that Applicants last explained that Safar *et al.* merely teach that heat or chemical treatment can have an effect on the degradation of a prion protein and the level of degradation can be measured by Western blot analysis, but Safar *et al.* do not teach or suggest a method of evaluating the efficiency of a sterilization process. Further, Safar *et al.* do not demonstrate any degradation by Western blot, *citing* Figure 1, allowing for the evaluation of the sterilization process. The Examiner replies that this is not a proper distinction because the current claims do not require that Western blotting show any degradation. The Examiner adds

that protein breakdown may be detected by other technology as well, e.g. CD spectrum.

Regarding Applicants' explanations that conformational change shown by Safar *et al.* is not degradation, the Examiner maintains that conformational change caused by thermal treatment is considered degradation. Further, the Examiner adds that the claims do not require that prion indicators be degraded, rather the claims are directed to determining whether there is any degradation

Regarding Applicants' explanations that Safar *et al.* do not measure degradation but rather measure conformational change, the Examiner replies that using Western blotting inherently provides information regarding whether there is degradation.

Regarding Applicants' explanations that there are significant differences between yeast prion proteins and mammalian proteins and one of ordinary skill in the art could not predict the utility of an invention based upon Safar *et al.* using the proteins of Coustou *et al.*, Glover *et al.*, or Wickner *et al.*, the Examiner says that this argument is not credible because it is not supported by evidence or declarations signed under oath. The Examiner cites Prusiner, *PNAS* (1998) already of record for the position that "prions" is extended to encompass yeast and other prions.

Regarding Applicants' explanations that ozone treatment is particularly effective and is not taught by Safar *et al.*, the Examiner replies that ozone treatment is not a limitation of the claims.

The Examiner seemingly invites Applicants to amend the claims in order to overcome the prior art rejections based upon obviousness. Further, the Examiner seemingly invites a declaration signed under oath explaining significant differences between yeast prion proteins and mammalian proteins or references in the literature explaining the same differences.

**Applicants' new amendments and explanations regarding patentability**

1) The claims are amended to require degradation.

Applicants reiterate that Safar *et al.* do not demonstrate, teach or suggest any degradation by Western blot or any other method. (See, Figure 1). To emphasize the fact that the method taught by Safar *et al.* is different from the method of the present invention, if Safar *et al.* had used a dot blot using antibody against the Histidine tail of the protein as suggested in one of the embodiments of the present invention, in their "denaturation process", Safar *et al.* would have still detected the protein. In clear opposition Applicants have shown in Fig. 5 that the protein

sought to be detected with the dot blot is not found or detected, clearly indicating physical degradation of the protein, not just denaturation. The process of Safar et al. would have been conclusive at best for one skilled in the art trying to reproduce the present invention with the process of Safar et al. that the sterilization process was inefficient because the protein, although denatured, would still be detected on such a dot blot. From the above and the amendments made to the claims presented herein, it is clear that the method taught in Safar et al. is different from the present invention in that Safar et al. do not teach or suggest degradation of the protein, let alone the fact that it is not even the same protein. Moreover, such deficiency is not taught or suggested in any of the references cited by the Examiner.

Additionally, one skilled in the art would have recognized that a denatured protein can refold and recover its original conformation, thus its infectivity. Refolding of proteins (with recovery of the activity) is a process known to occur. McKenzie *et al.* (*J. Biol. Chem.* 1998 Oct. 2; 273(40): 25545-7) (Exhibit C), in the abstract of their article published on PubMed recognized that infectivity of PrPSc following a denaturation, as evidenced by the loss of proteinase K resistance of PrPSc can be restored upon dilution or removal of the denaturing agent. (PubMed abstract enclosed). Moreover, Gao *et al.* (*Acta Virol.* 2006; 50(1): 25-32) (Exhibit D) confirmed in their abstract that “[o]n the other hand, the infectivity of PrP(Sc) inactivated by denaturation could be partially restored by renaturation.” In light of this, a person skilled in the art confronted with the problem of prion infection, especially in hospital, would have never considered a denaturation process, as it cannot guarantee that the prion is not infectious anymore or that the prion will not recover its infectivity over time. Therefore, one skilled in the art would not have considered Safar *et al.* as relevant prior art as a possible solution to the technical problem solved with the present invention.

It should be noted that rejecting the claims now for being allegedly obvious over Safar et al., can only be formulated following hindsight. In Safar *et al.*, the Western Blot does not show any degradation or reduction of the amount of protein measured, providing no information on the efficiency of the sterilization process. One skilled in the art upon reading Safar *et al.* would not have been led to a method for evaluating the efficiency of sterilization as now claimed, involving prion protein degradation.

2) The claims are amended to require that prion indicators are degraded.

Applicants reiterate that Safar *et al.* demonstrate only conformational change. Safar *et al.* do not demonstrate, teach or suggest degradation.

3) Applicants submit herewith a Second Declaration under 37 C.F.R. §1.132 of Pierre Belhumeur, Ph.D. (Exhibit B) further demonstrating significant differences between yeast prion proteins and mammalian proteins.

The scrapie amyloid prion protein (PrP<sup>27-30</sup>) used by Safar *et al.* is a mammalian prion protein, whereas those used in U.S. Patent Application Serial No. 09/980,649 are yeast prion proteins. Applicants reiterate that there are significant differences between yeast prion proteins and mammalian proteins and one of ordinary skill in the art could not predict the utility of an invention based upon Safar *et al.* using the proteins of Coustou *et al.*, Glover *et al.*, or Wickner *et al.* Supporting this allegation is a Declaration under 37 CFR § 1.132 from Dr. Pierre Belhumeur. In fact, because of these significant differences, any conclusion that can be made on mammalian prion proteins for example are not systematically transposable for yeast prion proteins. Among these significant differences, one skilled in the art will note that there is a low level of homology between the amino acid sequences of mammalian prion proteins and yeast prion proteins. For example, the Sup35 yeast prion protein contains 685 amino acids (Kushnirov *et al.*, *Gene* 66 (1), 45-54 (1988)) (Exhibit E) while the human prion protein PrP is 253 amino acids long (Kretzschmar *et al.*, *DNA* 5 (4), 315-324 (1986)) (Exhibit F). (*See*, Belhumeur Declaration §8)

Moreover, the yeast Sup35 protein binds GTP, is located in the cytosol and is a protein translation termination factor. On the other hand, the human prion protein PrP can bind copper, is a glycoprotein attached to the cell membrane and there are no indications of it being involved in protein translation. (*See*, Belhumeur Declaration §9) Just on the basis of these different properties, one skilled in the art would be led to believe that mammalian prion proteins would behave differently than yeast prion proteins and would never have been tempted to extrapolate results obtained on mammalian prion proteins to yeast prion proteins.

To even add more distinctions, the mechanisms of conversion of mammalian and yeast prion proteins into their infectious forms differ significantly. The formation of the infectious form of the human prion protein PrP occurs after it transits into a subcellular compartment such

as the lysosome while the change of conformation of the yeast prion proteins (e.g. Sup35) occurs in the cytoplasm. The mammalian prion protein changes conformation at pH 4.0 (the pH inside the lysosome) while the conformational changes for the yeast prion protein occurs at physiological pH (c. pH 7.4). (*See*, Belhumeur Declaration §10) The different pH at which a conformational change occurs suggests that the pI is also different, reflecting on different structures as well. Moreover, the conformational changes of the yeast prion proteins require a molecular chaperone (protein Hsp104, for the Sup35 yeast prion protein), but mammalian PrP prion proteins do not require any chaperone. (*See*, Belhumeur Declaration §12)

Still, as a further distinction mammalian prion aggregates are essentially made of fibrils (resistant to detergents), whereas there is no conclusive evidence that yeast prion proteins are essentially made of fibrils.

In light of the above, Applicants submit that these differences are significant and a person skilled in the art, knowing these differences (different physical properties), would have never concluded that mammalian prion proteins would behave the same way as yeast prion proteins do, such that treatment on mammalian prion proteins does not warrant the same results as that on yeast prion proteins.

4) Applicants add new claim 16 specifically reciting ozone treatment.

In order to further define particular embodiments of the invention, Applicants add new claim 16 specifically reciting ozone treatment. As such, Applicants reiterate that Safar *et al.* do not teach or suggest ozone treatment, which is particularly effective.

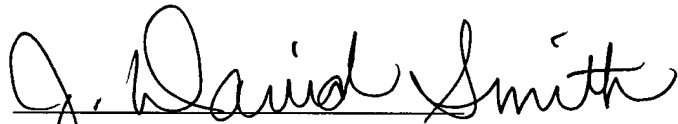
**FEES**

No additional fees are believed to be necessary. However, if any additional fees are due, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment.

**CONCLUSION**

Applicants respectfully request entry of the foregoing amendments and remarks in the file of the instant application. Early and favorable action in the form of a notice of allowance is earnestly solicited. If any issues remain, they may be resolved by telephone, the Examiner is invited to contact the undersigned at the telephone number provided below.

Respectfully submitted,

A handwritten signature in black ink, reading "J. David Smith". The signature is written in a cursive style with a large, looping initial "J".

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